



## Malformed frogs

### Background

Minnesota's malformed frog problem was first identified in 1995. Since then, Minnesota Pollution Control Agency (MPCA) staff have been working to find the cause of the problem, including collaborating with other researchers to establish and test hypotheses. Although malformed frogs now are found in many other states<sup>1</sup>, the MPCA effort focuses on Minnesota.

Since 1997, the agency, along with the National Institute of Environmental Health Sciences (NIEHS), the U.S. Geological Survey's Biological Resources Division (USGS), and the state departments of Natural Resources, Health, and Agriculture, have worked with a nationwide network of researchers to narrow the list of possible causes. The main theories revolve around chemical contaminants, parasites, and ultraviolet radiation. Many researchers believe there is no single explanation, and that these factors and perhaps others may be interrelated. Much of the MPCA's work on this project has been made possible by support from the Minnesota Legislature, including an initial LCMR grant and subsequent appropriations, and by significant contributions of time and money by our federal partners.

Beginning in 1998, frog surveys have been conducted at a group of study sites where frog malformation rates are high. Data on frog species, numbers, size, weight, and types of malformations are gathered. The USGS did diagnostic work on tadpoles and frogs, and the NIEHS is conducting extensive lab analyses of chemicals found in water and sediment, as well as biological assays. We also worked with the USGS to investigate the role that ground water may be playing in frog malformations at the sites.

### Recent findings

A California researcher made news in 1999 with a study that established the role of parasitic flatworms (*Ribeiroia*) in malformations of tree frogs in California. The MPCA has been looking at parasites as a potential cause for several years. Dr. Dan Sutherland, a parasitologist from the University of Wisconsin working with the MPCA, has found significant numbers of *ribeiroia* at only one Minnesota site, low numbers at another, and none at the other study sites. So far the parasite theory, while interesting, has not explained Minnesota's malformed frogs beyond possibly one site.

The MPCA has been collecting frogs at critical points in their development process and sending them to USGS, which is examining tissue to determine at what stage malformations begin to occur and what possible mechanisms might be triggering the malformations. This work should yield clues as to what type of developmental

<sup>1</sup> More than 40 states have reported malformed frogs since 1995. However, only a handful have reported large numbers of them, with Minnesota and Vermont seeming to have the most.





disturbance is occurring and what kinds of compounds might be causing the malformations. Results of this work have not been received yet.

We also continued to collect water and sediment samples at our study sites and sent them to NIEHS for testing and analysis.

## Progress on Minnesota research

Based upon the Minnesota team's efforts thus far, "natural" causes such as larval predation and infectious agents have been all but eliminated as possible causes of the widespread frog malformations observed in Minnesota. Parasites may be a factor at one of our study sites but apparently do not cause the malformations seen at other sites.

The list of potential causes developed by the Minnesota team to this point includes:

- The NIEHS has found that the water from study sites in Minnesota and Vermont causes malformed frogs in the laboratory, and that water from "normal" sites (no malformed frogs) does not. They have identified some of the chemicals in the water and have tested some of them for ability to cause frog malformations. The fungicide Maneb and propylthiourea, a pesticide, were shown to cause limb malformations. Several papers have been published on the results of this ongoing work.
- The thyroid gland plays a significant part in controlling frog metamorphosis. NIEHS recently reported that adding thyroid hormone to water and sediment extracts from study sites reduces the number of limb malformations observed in the lab, and adding anti-thyroid chemicals increases malformations. This suggests that one or more chemicals present at our study sites may be acting as endocrine disruptors and interfering with the normal development process. The MPCA has begun a special LCMR-funded project to measure vitellogenin in frogs at affected and normal sites, which will provide more clues on the potential role of endocrine disruption.
- The U.S. EPA and others have established that ultraviolet light, under laboratory conditions, can cause malformations in frogs. We are working with the USGS to measure ultraviolet light penetration at our study sites. They also are looking at how UV may be breaking down chemicals in the environment into other compounds which could be contributing to the malformations.

The MPCA continues to work with researchers examining frog tissue for certain types of damage that can provide clues to what is causing the malformations. For example, certain chemicals can interfere with limb formation or bone development in characteristic ways. Certain malformations, if present, may indicate that these chemicals are playing a role.

## Where do we go from here?

Using a 1999 legislative appropriation of \$600,000 for the current biennium, the MPCA will continue to review and synthesize data, participate in hypothesis formulation and testing, and contract with our research partners. We will continue to focus field and laboratory work on Minnesota sites, collecting and supplying samples for analysis, and synthesizing research results relevant to the problem in Minnesota.

## For more information

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